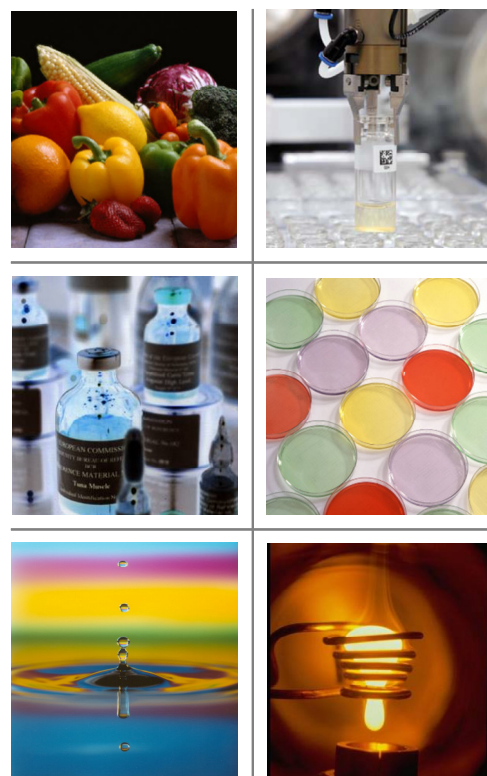


The Certification of the Mass Fractions of Tristearin in Blends of Pure Tristearin and Triolein Representing the Solid Fat Content IRMM-805 (Set of 12 Primary Standards)

A. Bernreuther, H. Schimmel



EUR 24959 EN - 2011

The mission of the JRC-IRMM is to promote a common and reliable European measurement system in support of EU policies.

European Commission
Joint Research Centre
Institute for Reference Materials and Measurements

Contact information

Address: Retieseweg 111, B2440 Geel, Belgium
E-mail: Alexander.bernreuther@ec.europa.eu
Tel.: +32 14 571 654
Fax: +32 14 571 548

<http://irmm.jrc.ec.europa.eu/>
<http://www.jrc.ec.europa.eu/>

Legal Notice

Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of this publication.

***Europe Direct is a service to help you find answers
to your questions about the European Union***

**Freephone number (*):
00 800 6 7 8 9 10 11**

(*) Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

A great deal of additional information on the European Union is available on the Internet.
It can be accessed through the Europa server <http://europa.eu/>

JRC 66999

EUR 24959 EN

ISBN 978-92-79-21470-7 (pdf)
ISBN 978-92-79-21469-1 (print)

ISSN 1831-9424 (online)

ISSN 1018-5593 (print)

doi:10.2787/53378

Luxembourg: Publications Office of the European Union, 2011

© European Union, 2011

Reproduction is authorised provided the source is acknowledged

Printed in Belgium

European Commission

IRMM information
REFERENCE MATERIALS

**The Certification of the Mass Fractions of
Tristearin in Blends of Pure Tristearin and
Triolein Representing the Solid Fat Content**

**IRMM-805
(Set of 12 Primary Standards)**

A. Bernreuther, H. Schimmel

European Commission,
Joint Research Centre
Institute for Reference Materials and Measurements
Retieseweg 111
2440 Geel, Belgium

Disclaimer

Certain commercial equipment, instruments, and materials are identified in this report to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the preparation and certification of IRMM-805. This reference material is comprised of a set of 12 tristearin/triolein blends (primary triglyceride samples) for the solid fat content (SFC) determination by low-resolution nuclear magnetic resonance spectrometry (LR-NMR).

The preparation and processing of the materials, stability studies and a characterisation study (by interlaboratory comparison) are described hereafter and the results are discussed. Uncertainties were estimated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [1] and include uncertainties due to gravimetric sample preparation, purity and possible instability. The certified values and their uncertainties are listed in Table I. In addition, indicative values of the corresponding F-factors and their uncertainties (as results from a characterisation study) are listed in Table II.

Table I: Certified solid fat contents (mass fractions) and their uncertainties for 12 triglyceride reference materials (IRMM-805)

Sample code	Solid fat content (SFC) [%] ¹⁾	Uncertainty [%] ²⁾
TGS-01	70.5	0.9
TGS-02	29.22	0.27
TGS-03	30.5	0.5
TGS-04	49.7	0.5
TGS-05	30.09	0.29
TGS-06	69.9	0.8
TGS-07	68.9	0.8
TGS-08	49.5	0.5
TGS-09	50.0	0.8
TGS-10	14.95	0.17
TGS-11	14.95	0.23
TGS-12	15.04	0.21

- 1) The certified values are expressed as the mass fractions of tristearin in blends of pure tristearin and triolein representing the solid fat content at 26 °C as specified in the measurement protocol (see Annex A). The certified values are traceable to the SI.
- 2) The certified uncertainty is the combined standard uncertainty with a coverage factor $k = 1$ corresponding to a level of confidence of about 68 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008

Table II: Indicative values for F-factors and their uncertainties for 12 triglyceride reference materials (IRMM-805)

Sample code	F-factor ¹⁾	Uncertainty ²⁾
TGS-01	1.561	0.024
TGS-02	1.567	0.021
TGS-03	1.573	0.031
TGS-04	1.594	0.029
TGS-05	1.568	0.021
TGS-06	1.568	0.025
TGS-07	1.571	0.027
TGS-08	1.582	0.025
TGS-09	1.581	0.030
TGS-10	1.569	0.023
TGS-11	1.575	0.029
TGS-12	1.569	0.028

- 1) As defined by the measurement protocol including prescribed instrumentation used for characterisation (see Annex A). The indicative values are based on the unweighted mean of means of 7 accepted sets of results.
- 2) The stated uncertainty is the combined standard uncertainty with a coverage factor $k = 1$ corresponding to a level of confidence of about 68 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008

Table of contents

Summary	1
Table of contents	2
Glossary	3
1 Introduction	4
2 Participants	5
3 Processing of the material	6
3.1 Material selection and preparation	6
3.2 Purity measurements	6
4 Homogeneity study	7
5 Stability studies	7
5.1 Short-term stability study	7
5.2 Long-term stability study	7
5.2.1 <i>Design of the long-term stability study</i>	7
5.2.2 <i>Results of the long-term stability study</i>	8
6 Characterisation study	9
6.1 Design	9
6.2 Results and technical evaluation	9
7 Certified values and uncertainties	11
8 Indicative values and uncertainties	12
9 Metrological traceability	13
10 Instructions for use and intended use	13
10.1 Safety precautions	13
10.2 Use of materials	13
10.3 Intended use	13
10.4 Storage conditions	13
11 Acknowledgements	14
12 References	14
Annex A Protocol for F-factor determinations of primary triglyceride standards (TGS) by low-resolution nuclear magnetic resonance spectrometry (LR-NMR)	15
Annex B Results of the long-term stability study	19
Annex C Characterisation study data	25

Glossary

α	Confidence level
AOCS.....	American Oil Chemists' Society
ANOVA.....	Analysis of variance
ASCII.....	American standard code for information interchange
b	Slope of linear regression
CAS.....	Chemical abstracts service
CL.....	Confidence level
CRM.....	Certified reference material
d_f	Degree of freedom (regression)
FAME.....	Fatty acid methyl ester
FID.....	Free induction decay (NMR)
GC-FID.....	Gas chromatography-flame ionisation detection
GLC.....	Gas liquid chromatography
GUM.....	Guide to the Expression of Uncertainty in Measurement
IRMM.....	Institute for Reference Materials and Measurements
ISO.....	International Organization for Standardization
JRC.....	Joint Research Centre of the European Commission
k	Coverage factor
KFT.....	Karl Fischer titration
l	Number of accepted data sets
LR-NMR.....	Low-resolution nuclear magnetic resonance spectrometry
n	Number of replicates
n.r.....	Not reported
OOO.....	Triolein (glycerol trioleate)
p	Level of significance
PIO.....	Plastic-in-oil standard
R^2	Coefficient of determination (regression)
RD.....	Recycle delay (NMR)
RSD.....	Relative standard deviation
RSD_{stab}	Relative standard deviation of all results of a stability study
RDE.....	Relative standard error
s	Standard deviation
se_b	Standard error of slope b of linear regression
SFC.....	Solid fat content
SI.....	International Systems of Units
SSS.....	Tristearin (glycerol tristearate)
t	Student's t -value
TGS.....	Triglyceride standard
$u_{char,rel}$	Relative standard uncertainty of characterisation study (interlaboratory comparison)
u_{CRM}	Combined standard uncertainty of certified value
$u_{CRM,rel}$	Combined relative standard uncertainty of certified value
$u_{lts,rel}$	Relative standard uncertainty of long-term stability
$u_{prep,rel}$	Relative standard uncertainty of sample preparation (including weighing and purity)
$u_{pur,rel}$	Relative standard uncertainty of purity
$u_{w,rel}$	Relative standard uncertainty of weighing
VT.....	Variable temperature (NMR)
x	Pre-defined shelf life
x_i	Result at time point i in a stability study
\bar{x}	Average result of all time points in a stability study

1 Introduction

Fast and reliable control during production of spreadable fats such as margarine is very important for food industry as consumers prefer products, which are at ambient temperatures sufficiently soft. Spreadability of such products can be assessed by the solid fat content (SFC). Although well-defined recipes are available, there might be significant variations of raw materials, which are not only influenced by crop varieties and extraction methods, but also by natural variations (climate, soil, fertilisation, etc.).

Traditionally, SFC was determined by dilatometry (laborious, empiric measurement of the expansion of the fat upon heating in a calibrated tube) [2]. The current method of choice is the fast and robust direct SFC method (Cd 16b-93) based on low-resolution nuclear magnetic resonance spectrometry (LR-NMR), which has been accepted by the AOCS (American Oil Chemists' Society) [3]. In 2008, also a corresponding ISO standard was released (ISO 8292-1) [4]. As SFC is temperature dependent, SFC measurements have to be performed at a defined temperature, which is in case of the direct LR-NMR method 26 °C.

The direct SFC method requires a system of reference materials, which consists of a set of primary standards and a set of secondary standards with certified solid fat contents. They are used to calculate a correction factor (F-factor), which accounts for a dead time in the electronics of LR-NMR spectrometers. The secondary standards are intended for daily use. They usually consist of a set of three robust and stable plastic-in-oil (PIO) samples (SFC = 0 %, ~ 30 % and ~ 70 %). These PIOs are prepared in a manner that is considered “*proprietary*” and are provided by the corresponding instrument manufacturer.

The design of the PIOs is based on an arbitrarily chosen blend of oils and fats from the late 1970's, with known SFCs. Up to now, PIOs for daily use in the laboratories are linked to a PIO master batch, which is kept with the respective instrument manufacturer. It is important to mention that there is a significant difference between PIOs provided by Bruker BioSpin (Rheinstetten, DE) and by Oxford Instruments (Abingdon, UK), which are the main producers of LR-NMR instruments. In order to be able to compare SFC results worldwide, a harmonised approach was demanded by the AOCS to establish a reference system based on primary standards, which have to be independent from the instruments used, and thus independent from instrument manufacturers.

Therefore, the IRMM as an independent institute was contacted to gravimetrically produce blends of tristearin (glyceryl tristearate; SSS; CAS number 555-43-1; melting point: 72 °C) and triolein (glyceryl trioleate; OOO; CAS number 41755-78-6; melting point: 5 °C) of high purity (sealed NMR tubes). These triglycerides can be obtained from independent sources and are renewable (sustainable). At ambient temperature SSS is considered to be solid and OOO to be liquid, thus the SFC of such blends is equal to the amount of SSS. In total, 3 sets each comprising of 4 triglyceride standards (TGSs) were produced (with nominal SFCs of 15 %, 30 %, 50 % and 70 %). This set of materials is intended to be exclusively used as primary standards for calibration of secondary standards, thus is not intended for use outside of the IRMM.

Another major goal of the study was to demonstrate that the calculated F-factors after measurement of each sample of IRMM-805 were independent from the SFC of the respective triglyceride sample and from the instruments used. For confirmation these TGS samples were submitted to a characterisation study, which was set up as a “*sequential*” interlaboratory comparison, where all samples (= 12 samples) were measured by 11 laboratories from Europe and North America. The measurements were performed on instruments from three different manufacturers, following a strict protocol (see Annex A).

2 Participants

Project management and evaluation

European Commission (EC), Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), Reference Materials Unit, Geel, BE

Processing

European Commission (EC), Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), Reference Materials Unit, Geel, BE

Stability measurements

European Commission (EC), Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), Reference Materials Unit, Geel, BE

Characterisation measurements (interlaboratory comparison)

Bruker BioSpin GmbH, Rheinstetten, DE

European Commission (EC), Joint Research Centre (JRC), Institute for Reference Materials and Measurements (IRMM), Reference Materials Unit, Geel, BE

Friesland Coberco Dairy Foods, Deventer, NL

Kraft Foods, Glenview, IL, USA

Oxford Instruments, Abingdon, UK

Pennsylvania State University, Dept. Food Science, University Park, PA, USA

Resonance Instruments Ltd., Witney, UK

Ryerson University, Dept. Nutrition, Toronto, ON, CA

Unilever Research Vlaardingen, Vlaardingen, NL

University of Alberta, Dept. Agriculture, Food and Nutritional Sciences, Edmonton, AB, CA

University of Guelph, Dept. Food Science, Guelph, ON, CA

3 Processing of the material

3.1 Material selection and preparation

Triolein and tristearin with purities > 99 % (indicated on the certificates of analysis based on chromatographic methods) were purchased from Sigma-Aldrich NV/SA (Bornem, BE). Glass NMR tubes (h = 18 cm; diameter = 10 mm) were purchased from Resonance Instruments Ltd. (Witney, UK). In total, 12 blends of SSS and OOO were gravimetrically prepared and filled into NMR tubes at the IRMM (three samples per SFC level; see Table 1).

In addition, three NMR tubes with pure OOO and three empty NMR tubes were prepared, which were needed for the F-factor calculation (for details see Section 6). Approximate filling height of each NMR tube is 5.5 ± 0.1 cm. All NMR tubes were carefully flame-sealed under inert gas (argon).

Samples with 30 % and 70 % SFC were chosen to imitate typically used secondary standards (PIOs). To ensure more detailed information over the whole SFC range, also 15 % and 50 % SFC were chosen as the mean of pure oil (0 % SFC) and 30 % SFC as well as the mean of 30 % and 70 % SFC, respectively. Measurement uncertainties derived from preparation (weighing) are presented in Section 7.

Table 1: Quantities of tristearin (SSS) and triolein (OOO) used for preparation of 12 NMR tubes

Sample code	Nominal SFC [%]	Amount SSS [g]	Amount OOO [g]	Total amount [g]	Final SFC [%]
TGS-01	70	1.5801	0.6598	2.2399	70.543
TGS-02	30	0.6743	1.6335	2.3078	29.218
TGS-03	30	0.6941	1.5810	2.2751	30.509
TGS-04	50	1.1248	1.1384	2.2632	49.700
TGS-05	30	0.6782	1.5755	2.2537	30.093
TGS-06	70	1.5751	0.6793	2.2544	69.868
TGS-07	70	1.5746	0.7116	2.2862	68.874
TGS-08	50	1.1252	1.1483	2.2735	49.492
TGS-09	50	1.1238	1.1238	2.2476	50.000
TGS-10	15	0.3393	1.9305	2.2698	14.948
TGS-11	15	0.3381	1.9234	2.2615	14.950
TGS-12	15	0.3386	1.9130	2.2516	15.038

3.2 Purity measurements

The purity of SSS and OOO was assessed by volumetric Karl Fischer titration (KFT) [5] as well as determination of fatty acid methyl ester content (FAME) and direct triglyceride analysis by gas chromatography-flame ionisation detection (GC-FID) [6,7].

The water contents of SSS and OOO were found to be below the limit of quantification (0.05 g/100 g). The GC analysis of SSS and OOO indicated purities of > 99.5 %, which was considered to be sufficient for the purpose of this study. An even more detailed knowledge on impurities (with much more effort) would not support the calculation of the real solid fat content at room temperature, thus a rather conservative approach was chosen to account for the measurement uncertainty derived from purity determinations (see Section 7).

4 Homogeneity study

No homogeneity study was performed as all 12 prepared triglyceride standards are certified for its solid fat content.

As the tube as a whole has to be measured in the LR-NMR spectrometer, the minimum sample intake issue is not applicable here. Nevertheless, it is important that the NMR tubes are correctly inserted into the spectrometers as specified by the instrument manufacturers.

5 Stability studies

5.1 Short-term stability study

No short-term stability study was performed because all triglyceride samples were submitted to the long-term stability study. Moreover, the measurement protocol requires heating of the samples up to 80 °C for at least 15 min per measurement cycle, which was done repeatedly without any impact on the samples (see results from long-term stability study below). Finally, the set of materials is intended to be exclusively used as primary standards for calibration of secondary standards for solid fat content (SFC) determinations.

5.2 Long-term stability study

5.2.1 Design of the long-term stability study

In order to assess the stability of the triglyceride samples (TGS-01 to TGS-12), F-factors were determined in triplicate at the start of the interlaboratory comparison, in the middle (5 months later) and after 27 , 47 and 93 months following the same measurement protocol as has been followed for the characterisation study (see Annex A and Section 6). Between periods of transports and measurements, the samples were continuously stored at 18 °C. This temperature was chosen based on experience with triglycerides as well as on the fact that controlled cooled shipping to overseas participants would have been too difficult to organise (samples were sent from participant to participant, which was out of control for IRMM).

Grubbs tests at 99 % confidence levels (CL) were performed to detect potentially outlying results. Data points were plotted against time and the regression lines were calculated to check for significant trends (degradation, enrichment) due to storage conditions (see Table 2 for a summary). The observed slopes (b) were tested for significance using a t -test, with $t_{\alpha,df}$ being the critical t -value (two-tailed) for a confidence level $\alpha = 0.05$ (95 % confidence level) and for a confidence level $\alpha = 0.01$ (99 % confidence level). The slope was considered as statistically significant when $|b|/se_b > t_{\alpha,df}$. Finally, the uncertainty of stability $u_{lts,rel}$ [8] was calculated for a pre-defined shelf life of 108 months applying equation 1. The stability of the samples will be further monitored on a regular basis.

$$u_{lts,rel} = \frac{RSD_{stab}}{\sqrt{\sum (x_i - \bar{x})^2}} \cdot x \quad (1)$$

with RSD_{stab} being the relative standard deviation of all individual results ($n = 15$) of the relevant stability study, x_i being the time point for each replicate, \bar{x} being the average of all time points and x being the pre-defined shelf-life. Graphs can be found in Annex B.

5.2.2 Results of the long-term stability study

One data set for TGS-11 was missing (run 1, 27 months). One outlier (individual results; single Grubbs test) was detected each for TGS-03 as well as for TGS-09. In both cases no technical reasons for exclusion could be given. Therefore, entire data sets were kept to calculate the respective $u_{\text{ts,rel}}$.

For none of the samples a significant slope (95 % confidence level) was detected, which demonstrates the stability of the material under these conditions. As storage temperature for all triglyceride samples $18\text{ °C} \pm 5\text{ °C}$ was chosen (ambient temperature).

Table 2: Evaluation of 93 months long-term stability study at 18 °C for triglyceride reference materials

Sample code	TGS-01	TGS-02	TGS-03	TGS-04	TGS-05	TGS-06	TGS-07	TGS-08	TGS-09	TGS-10	TGS-11	TGS-12
SFC [%]	70.54	29.22	30.51	49.70	30.09	69.87	68.87	49.49	50.00	14.95	14.95	15.04
F-factor	1.561	1.567	1.573	1.594	1.568	1.568	1.571	1.582	1.581	1.569	1.575	1.569
$ b /se_b$	1.35	1.48	0.09	0.94	0.90	1.11	0.92	1.40	0.51	0.96	1.73	0.78
Outlier test (Grubbs) (99 % CL)	none	none	1	none	none	none	none	none	1	none	none	none
Statistical significance of the slope (95 % CL) ¹⁾	no	no	no	no	no	no	no	no	no	no	no	no
Statistical significance of the slope (99 % CL) ²⁾	no	no	no	no	no	no	no	no	no	no	no	no
$u_{\text{ts,rel}}$ [%/108 months]	0.50	0.38	1.36	0.31	0.45	0.41	0.54	0.25	1.17	0.63	1.20	1.07

1) $t_{0.05;10} = 2.160$

2) $t_{0.01;10} = 3.012$

6 Characterisation study

6.1 Design

A sequential interlaboratory comparison was launched in 2003 to demonstrate that the calculated F-factors after measurement of each sample of IRMM-805 were independent from the SFC of the respective triglyceride sample and from the instruments used. Eleven experienced laboratories from industry and research were selected to perform the SFC measurements. All 12 TGS samples (NMR tubes) were sent from participant to participant, and finally returned to IRMM. The laboratories were asked to perform F-factor determinations strictly following the “Protocol for F-factor determinations of primary triglyceride standards (TGS) by low-resolution nuclear magnetic resonance spectrometry (LR-NMR)” (see Annex A) and to deliver raw data to IRMM, where finally F-factor calculations were performed. Each TGS sample had to be measured in triplicate on three different days, each time after a special tempering procedure (in total more than 40 h; for details see Annex A). Each laboratory had to send three sets of raw data per TGS sample. Instrument and method settings are summarised in Table 3.

Table 3: Instrument and method settings used for SFC measurements for IRMM-805

Lab code	Instrument manufacturer and typed	Pulse length ¹⁾ [μs]	Dead time ²⁾ [μs]	First signal ³⁾ [μs]	Sampling frequency [MHz]	Flushing of tempering blocks with N ₂	Use of sampling robots for the tempering scheme
1	Bruker minispec mq-20	2.70	7.00	10.64	2.5	N	N
2	Bruker minispec mq-20	2.64	7.00	10.84	2.5	Y	N
3	Bruker minispec mq-20	2.70	7.00	10.64	2.5	N	N
4	Resonance Maran Ultra	3.05	6.95	10.40	2.5	N	N
5	Resonance Maran Ultra	3.20	6.80	10.80	2.5	N	N
6	Bruker minispec mq-20	2.62	7.00	10.54	2.5	N	N
7	Bruker minispec mq-20	2.56	7.00	10.84	2.5	Y (but with air)	Y
8	Resonance Maran Ultra	2.60	7.15	10.55	5.0	N	N
9	Bruker NMS120	2.85	7.00	10.00	10.0	N	N
10	Oxford QP20	10.40 (total)		11.60	2.5	n.r.	n.r.
11	Bruker minispec mq-20	2.30	9.30	12.14	2.5	N	N

1) Pulse length = 90° pulse duration

2) Sum of probe head ring down time and receiver dead time

3) Recorded time of first signal, which could be used for linear regression to calculate F-factors (see Section 6.2)

4) n.r. = not reported

6.2 Results and technical evaluation

After receipt of the data sets from each laboratory, method and instrument parameters as well as raw data were technically evaluated. Raw data were then subjected to F-factor calculations (see equation 2) using a validated software package (based on MS[®] Excel; ACOMED statistik, Leipzig, DE).

$$F = \frac{\text{SFC} \cdot (E_{45-55} + D_{45-55})}{(100 - \text{SFC}) \cdot (E_{11} \cdot K - E_{45-55})} \quad (2)$$

E_{11} = solid signal at 11 μs

E_{45-55} = liquid signal averaged between 45 μs and 55 μs

D_{45-55} = offset of zero-signal (empty tube) averaged between 45 μs and 55 μs

K = ratio of averaged liquid signal over solid signal of a pure triolein sample (E_{45-55}/E_{11})

To account appropriately for varying starting times applied by participants and quick decays of the signals around 11 μ s, several options were tested using the above-mentioned software package. It was determined (based on best R^2 -values) that linear regression using six data points around 11 μ s lead to best interpolations of E_{11} . For E_{45-55} 26 data points were used.

All data sets from Labs 8 and 9 were excluded because sample frequencies different from the specified 2.5 MHz were used (see Table 3). Also all data sets from Labs 10 and 11 were excluded as they reported instrument dead times larger than 10 μ s, while a total dead time (incl. pulse length) ≤ 10 μ s was requested. This is crucial as in most cases the first two or three recorded signals cannot be used for interpolation of E_{11} via linear regression. Therefore, it was a prerequisite for correct F-factor calculations that at least one valid signal was recorded before 11 μ s. In total, data sets from eight laboratories were accepted (= 3 x 8 = 24 results per TGS sample) and submitted to the following statistical tests:

- Grubbs test to detect single and double outliers
- Cochran test to check for outlying laboratory variances
- Skewness and kurtosis tests to assess the normality of the lab means distribution

The results of the statistical tests of the finally considered F-factors for all TGS samples of IRMM-805 are summarised in Table 4. Individual results and corresponding graphs can be found in Annex C. In all cases, variances between labs were significantly different (Snedecor *F*-test), therefore data could not be pooled and had to be grouped by labs. Moreover, it was decided to keep those data sets, which have outlying laboratory variances (Cochran test) as no technical reasons could be given for their exclusion. For TGS-01 one laboratory provided only two data sets (run 2 was missing), thus in total only 20 data sets were obtained.

Finally, no conclusions can be drawn, whether the use of sampling robots and/or flushed tempering blocks might improve the measurements as only one respectively two laboratories applied such techniques.

Table 4: Summary of statistical evaluation of F-factor determinations for 12 triglycerides (IRMM-805)

Sample code	TGS-01	TGS-02	TGS-03	TGS-04	TGS-05	TGS-06	TGS-07	TGS-08	TGS-09	TGS-10	TGS-11	TGS-12
SFC [%]	70.54	29.22	30.51	49.70	30.09	69.87	68.87	49.49	50.00	14.95	14.95	15.04
Number of accepted data sets, <i>l</i>	7	7	7	7	7	7	7	7	7	7	7	7
Number of replicate measurements	20	21	21	21	21	21	21	21	21	21	21	21
Mean of means	1.561	1.567	1.573	1.594	1.568	1.568	1.571	1.582	1.581	1.569	1.575	1.569
Relative std. deviation of mean of means [%]	2.53	2.56	3.01	4.13	2.46	2.98	3.27	3.36	3.20	2.49	2.59	2.79
* Relative std. error of mean of means [%]	0.96	0.97	1.14	1.56	0.93	1.13	1.24	1.27	1.21	0.94	0.98	1.05
Outlying lab means? (Grubbs test; $p = 0.05$)	none	none	none	none	none	none	none	none	none	none	none	none
Outlying lab variances? (Cochran test; $p = 0.05$)	yes (L3)	none	yes (L1)	yes (# L1) (L2) (# L6)	none	yes (# L7)	yes (# L7)	none	yes (L1) (# L6)	none	none	none
Distribution of means normal ($p = 0.05$)? (Skewness, kurtosis and normal probability plot)	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes

* relative standard error of mean of means = RSD/\sqrt{l}

$p = 0.01$

7 Certified values and uncertainties

The certified values for the 12 primary triglyceride reference materials (IRMM-805) are expressed as the mass fractions of tristearin in blends of pure tristearin and triolein representing the solid fat content and are based on gravimetric preparation (see Table 1).

The gravimetric values were indirectly confirmed by the results of the characterisation study showing that the obtained F-factors are independent from both, the solid fat content of the triglyceride standards and the instruments used (for details see Section 8).

The combined standard uncertainty of the certified value includes contributions from sample preparation, purity and long-term storage. The relative combined standard uncertainty is calculated according to equation 3:

$$u_{\text{CRM,rel}} = \sqrt{u_{\text{prep,rel}}^2 + u_{\text{ts,rel}}^2} \quad (3)$$

The uncertainty contribution from the gravimetric sample preparation was based on two weighing steps per TGS sample (one weighing step for SSS and one weighing step for OOO). Per weighing step a relative standard uncertainty ($u_{\text{w,rel}}$) of 0.050 % was reported.

The uncertainty contribution from the purity ($u_{\text{pur,rel}}$) of the triglycerides was estimated in a rather conservative manner and set to 1 % for each compound to account for all possible impurities (see Section 3.2).

The uncertainty contributions from weighing and purity ($u_{\text{w,rel}}$ and $u_{\text{pur,rel}}$) were combined according to the GUM [1]. The resulting relative combined uncertainties ($u_{\text{prep,rel}}$) are listed in Table 5.

A coverage factor of $k = 1$ was chosen as the set of triglyceride reference materials (IRMM-805) will exclusively serve as primary standards for the calibration of secondary standards.

Table 5 summarises the individual uncertainty contributions and the combined relative uncertainties as well as the certified values and their absolute uncertainties after rounding for all TGS samples.

Table 5: Certified values and uncertainties for 12 triglyceride reference materials (IRMM-805)

Sample code	TGS-01	TGS-02	TGS-03	TGS-04	TGS-05	TGS-06	TGS-07	TGS-08	TGS-09	TGS-10	TGS-11	TGS-12
$u_{\text{prep,rel}}$ [%]	1.042	0.821	0.819	0.865	0.819	1.034	1.024	0.864	0.867	0.877	0.877	0.877
$u_{\text{ts,rel}}$ [%] ¹⁾	0.496	0.379	1.358	0.308	0.450	0.409	0.538	0.247	1.170	0.635	1.202	1.067
$u_{\text{CRM,rel}}$ [%]	1.15	0.90	1.59	0.92	0.93	1.11	1.16	0.90	1.46	1.08	1.49	1.38
Certified value (SFC) [%]	70.5	29.22	30.5	49.7	30.09	69.9	68.9	49.5	50.0	14.95	14.95	15.04
u_{CRM} ($k = 1$) [%]	0.9	0.27	0.5	0.5	0.29	0.8	0.8	0.5	0.8	0.17	0.23	0.21

1) Shelf life 108 months

8 Indicative values and uncertainties

The indicative values for the F-factors for the 12 primary triglyceride reference materials (IRMM-805) are calculated as the mean of means of the accepted data sets. The standard error of the mean of means was used as an estimation of the uncertainty contribution of the characterisation study. The standard error is calculated as the standard deviation divided by the square root of the number of accepted data sets.

The combined standard uncertainty of the indicative value includes contributions from the certified value of SFC ($u_{\text{CRM,rel}}$; see Section 7) as well as of the characterisation study ($u_{\text{char,rel}}$). The relative combined standard uncertainty is calculated according to equation 4:

$$u_{\text{Ind,rel}} = \sqrt{u_{\text{CRM,rel}}^2 + u_{\text{char,rel}}^2} \quad (4)$$

A coverage factor of $k = 1$ was chosen as the set of triglyceride reference materials (IRMM-805) will exclusively serve as primary standards for the calibration of secondary standards.

Table 6 summarises the individual uncertainty contributions and the combined relative uncertainties as well as the indicative values and their absolute uncertainties after rounding for all TGS samples.

Table 6: Indicative values and uncertainties for 12 triglyceride reference materials (IRMM-805)

Sample code	TGS-01	TGS-02	TGS-03	TGS-04	TGS-05	TGS-06	TGS-07	TGS-08	TGS-09	TGS-10	TGS-11	TGS-12
$u_{\text{CRM,rel}}$ [%]	1.153	0.904	1.586	0.919	0.935	1.112	1.157	0.899	1.456	1.083	1.488	1.381
$u_{\text{char,rel}}$ [%]	0.958	0.968	1.138	1.562	0.929	1.126	1.236	1.270	1.208	0.941	0.977	1.053
$u_{\text{Ind,rel}}$ [%]	1.50	1.32	1.95	1.81	1.32	1.58	1.69	1.56	1.89	1.43	1.78	1.74
Indicative value (F-factor)	1.561	1.567	1.573	1.594	1.568	1.568	1.571	1.582	1.581	1.569	1.575	1.569
$u_{\text{Ind}} (k = 1)$	0.024	0.021	0.031	0.029	0.021	0.025	0.027	0.025	0.030	0.023	0.029	0.028

1) Shelf life 108 months

One of the major goals of the characterisation study was to demonstrate that the calculated F-factors after measurement of each sample of IRMM-805 were independent from the SFC of the respective triglyceride sample and from the instruments used. To verify whether there were significant differences of the F-factors between the triglyceride samples, they were grouped according to their four SFC levels, but also as an entire group and submitted to ANOVA (analysis of variance). For none of the groups significant differences were found. The results (incl. means and standard deviations) are summarised in Table 7.

Table 7: Results of ANOVA on F-factors grouped by four different SFC levels and as entire group

SFC level	15 %	30 %	50 %	70 %	All levels
number of samples	3	3	3	3	12
mean [F-factor]	1.571	1.569	1.586	1.567	1.573
standard deviation [F-factor]	0.039	0.040	0.054	0.044	0.045
RSD [%]	2.50	2.56	3.43	2.81	2.84
significant difference?	No	No	No	No	No

9 Metrological traceability

All triglyceride standards were gravimetrically prepared using calibrated balances. The assigned mass fractions of tristearin in blends of pure tristearin and triolein representing the solid fat content are traceable to the SI.

10 Instructions for use and intended use

10.1 Safety precautions

The usual laboratory safety precautions apply.

10.2 Use of materials

Before use the materials have to be subjected to a tempering procedure as specified in the measurement protocol (see Annex A).

10.3 Intended use

The NMR tubes have to be measured as a whole in the LR-NMR spectrometer. The set of materials is intended to be exclusively used as primary standards for calibration of secondary standards for solid fat content (SFC) determinations.

10.4 Storage conditions

The materials should be stored at ambient temperature ($18\text{ °C} \pm 5\text{ °C}$).

11 Acknowledgements

The authors would like to thank G. Buttinger and G. Roebben (IRMM, BE) for internal review of this report as well as P. Gowik (Federal Office of Consumer Protection and Food Safety, DE), P. Finglas (Institute of Food Research, UK) and J. de Boer (Institute for Environmental Studies, VU University Amsterdam, NL) as members of the Certification Advisory Panel for reviewing the certification documents and for their constructive comments.

12 References

1. International Organisation for Standardization, ISO/IEC Guide 98-3: Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995) (2008), ISO, Geneva, CH.
2. American Oil Chemists' Society, AOCS Official Method Cd 10-57: Solid fat index -- Dilatometric method (1997), AOCS, Urbana, IL, USA.
3. American Oil Chemists' Society, AOCS Official Method Cd 16b-93: Solid fat content (SFC) by Low-Resolution Nuclear Magnetic Resonance -- The direct method (1999), AOCS, Urbana, IL, USA.
4. International Organisation for Standardization, ISO 8292-1: Animal and vegetable fats and oils -- Determination of solid fat content by pulsed NMR -- Part 1: Direct method (1997), Geneva, CH.
5. Kestens, V., Conneely, P., Bernreuther, A. Vaporisation coulometric Karl Fischer titration: A perfect tool for water content determination of difficult matrix reference materials. Food Chem. 106 (2008) 1454.
6. American Oil Chemists' Society, AOCS Official Method Ce 1e-91: Determination of fatty acids in edible oils and fats by capillary GLC (1997), AOCS, Urbana, IL, USA.
7. American Oil Chemists' Society, AOCS Official Method Ce 5-86: Triglycerides by gas chromatography (1997), AOCS, Urbana, IL, USA.
8. Linsinger, T., Pauwels, J., Lamberty, A., Schimmel, H., van der Veen, A.M.H., Siekmann, L. Estimating the uncertainty of stability for matrix CRMs. Fres. J. Anal. Chem. 370 (2001) 183.

Annex A Protocol for F-factor determinations of primary triglyceride standards (TGS) by low-resolution nuclear magnetic resonance spectrometry (LR-NMR)

Definition

The measurement protocol described thereafter is based on the AOCS Official Method Cd 16b-93 (1999) "Solid fat content (SFC) by Low-Resolution Nuclear Magnetic Resonance – The direct method".

Apparatus

Most of the requirements are as according to AOCS Official Method Cd 16b-93. Please take note of amendments listed below. Important parameters are shown as underlined.

NMR Instrumentation

The NMR measurements can be performed by a low-resolution pulsed NMR instrument that is capable of measuring the signal from the solid part of the sample. The instrument should operate at a field strength of at least 0.47 T (or a Larmor frequency of protons > 20 MHz). The instrument must have a dead time (pulse duration + probehead ringdown time + receiver dead time) of 10 μ s or less. The instrument should also have a variable repetition delay between pulses (also known as trigger time). The instrument should be operated and maintained under the conditions outlined in AOCS Official Method Cd 16b-93.

NMR Measurement

The free induction decay (FID) following the pulse should be

- sampled in quadrature mode
- with a sampling frequency of 2.5 MHz
- and 16 scans (accumulations) plus 1 dummy scan
- with a repetition time (trigger time) of 25 s
- between the end of the dead time (pulse duration + probehead ringdown time + receiver dead time) and 100 μ s after the beginning of the radio frequency pulse
- it should be recorded on disk in a three-column ASCII format (time, channel A, channel B)
- the temperature of the probe head should be maintained at 26.0 ± 0.1 °C (a variable temperature (VT) probe head should be used)
- the probe head in the magnet gap should be suitable for sample tubes of 10 mm diameter

Sample Tempering – Constant Temperature Water Baths or Heating Blocks

The dimensions/specifications of the water baths/heating blocks are as according to AOCS Official Method Cd 16b-93. When the blocks are operated below 20 °C, the holes should preferably be flushed with nitrogen gas to prevent water condensation. The temperature of the gas should be preconditioned at the temperature of the bath. It should be mentioned with the results, if the blocks are not flushed. The analyst should be sure that the level of the sample is at least 5 mm below the outside surfaces of the aluminum tempering blocks.

Tempering Procedure

Rationale

Tristearin/triolein (SSS/OOO) blends exhibit polymorphic crystal behavior of the solid tristearin component. Crystalline tristearin can occur in metastable α - and β' -forms, but the most stable form is the β -polymorph. Typically, the α -form is formed directly after rapid cooling of the molten state. At room temperature the α -form converts to the β -form, which typically takes 40 h. In order to bring tristearin in a stable β -modification, this protocol uses a slightly modified version of the “Stabilizing Confectionery Fats” procedure (METHOD II in AOCS Official Method Cd 16b-93). The protons in the stable β -crystal form of tristearin in SSS/OOO blends possess NMR properties that are non-typical for blends used in industrial practice.

- Firstly, one can observe a periodic ‘beat’ pattern in the FID, which can be attributed to the strong dipolar couplings in the rigid tristearin crystal lattice (see Figure A1). Participants should not get confused by the non-Gaussian appearance of the ‘solid’ decay signal.
- Secondly, tristearin protons recover relatively slowly from the radio frequency pulse applied, i.e. a long T_1 relaxation time is observed. For this reason a pulse repetition time of 25 s should be used. This is a main reason for using a VT probehead, thermostated at 26 °C. In a non-VT probehead the sample would undergo (partial) melting.

Checking the Instrument and Tempering Blocks

This check is to be run at the beginning of each measurement period to detect possible instrument failures and wrong temperature settings. The analyst should be sure that the level of the sample is at least 5 mm below the outside surfaces of the aluminum tempering blocks.

Measuring and Handling Procedure

The tempering procedure is based on the “Stabilizing Confectionery Fats” procedure (METHOD II in AOCS Official Method Cd 16b-93). Deviations from this procedure at underlined:

- Melt the samples in a rack in the 80 °C water bath^{1,2} and keep them there for at least 5 min but not longer than 15 min. Homogenize the samples thoroughly and place them back in the rack for 5 min.
- Place the tubes in the 60 °C bath for 5 min.
- After tempering the tubes at 60 °C, place them in a rack in the 0 °C bath. Keep them there for 90 ± 5 min.
- Transfer the tubes to the 26 °C bath and keep them there for 40 ± 0.5 h.
- Transfer the tubes back to the 0 °C bath and keep them there for 90 ± 5 min.
- Remove the sample tubes from the 0 °C bath and place them in the block of the water bath at 26 ± 0.2 °C.
- Remove the dry tube from the block after 60 - 150 min³ and transfer it as quickly as possible into the sample holder of the NMR instrument, ensuring that the tube touches the bottom of the holder.
- A pulse repetition (trigger) time (recycle delay [RD]) of 25 s is used. Free induction decays are accumulated using 16 scans plus 1 dummy scan. Acquisition of the free induction decays should start at exactly 10 μ s until 100 μ s (with $t = 0$ being the beginning of the radio frequency pulse).

Measurement Scheme

General notes:

1. Samples should be measured in the order that is specified by IRMM.

¹ In order to ensure that no degradation effects can occur during the study, samples are melted at 80 °C instead of 100 °C.

² In place of a water bath an oven at 80 °C could be used for melting of the samples (full melting must be assured).

³ The tolerance of the residence time in the 26 °C block has been increased (from 60 ± 5 to 60 - 150 min) in order to facilitate efficient measurement of the sample series.

2. The scheme in Figure 2 can be used for rapid and effective measurement of the sample series, if no sampling robots are available.
3. Three sets of 15, 30, 50 and 70 % primary triglyceride standards should be tempered and measured in triplicate under the conditions specified previously. These measurements should be carried out by at least six different laboratories.
4. In order to calculate the K-factor three tubes filled with pure triolein (delivered with the shipment) should be measured in triplicate. No tempering procedure is needed, but all previously specified conditions pertain.
5. In order to detect (and correct for) offset effects three empty tubes (delivered with the shipment) should be measured in triplicate. No tempering procedure is needed, but all previously specified conditions pertain.
6. The primary triglyceride standards should be stored at ambient temperature, if not measured or tempered.
7. As a reference, three sets of 30 % and 70 % secondary plastic-in-oil standards (PIO) should be measured in triplicate. Except for a temperature equilibration for at least 30 min at 26 °C, no tempering procedure is needed, but all previously specified conditions pertain.
8. The three sets of 30 % and 70 % secondary plastic-in-oil standards should be measured again in triplicate, but using a pulse repetition (trigger) time (recycle delay [RD]) of 2 s and accumulating the free induction decay using 4 scans. Except for a temperature equilibration for at least 30 min at 26 °C, no tempering procedure is needed, but all previously specified conditions pertain.
9. The secondary plastic-in-oil standards should be stored upright at ambient temperature. In the case bubbles can be observed in these samples (e.g. caused by transportation), wait some days until the bubbles disappeared.

Record the following data and send them to the organizer of the collaborative study:

- complete quadrature FIDs in a three-column ASCII format (time and two signal axes [channel A and channel B])
- date of measurement
- sample code, as specified by IRMM
- NMR instrument manufacturer
- pulse length
- dead time (pulse duration + probehead ring down time + receiver dead time)
- flushing of tempering blocks with nitrogen (Y/N)
- use of sampling robots for the tempering scheme (Y/N)

Data Processing

Calculations

Note: The organizer of the collaborative study will perform the F-factor calculations. No effort of the participants is needed.

The signal should be sampled at two intervals:

E_{11} = solid signal at 11 μ s

E_{45-55} = liquid signal averaged between 45 μ s and 55 μ s

K = ratio of averaged liquid signal over solid signal of a pure triolein sample (E_{45-55}/E_{11})

D = offset of zero-signal (empty tube) averaged between 45 μ s and 55 μ s

The F-factors for every sample should be calculated according to:

$$F = \frac{\text{SFC} \cdot (E_{45-55} + D_{45-55})}{(100 - \text{SFC}) \cdot (E_{11} \cdot K - E_{45-55})}$$

NOTES

Caution

The same safety measures as in AOCS Official Method Cd 16b-93.

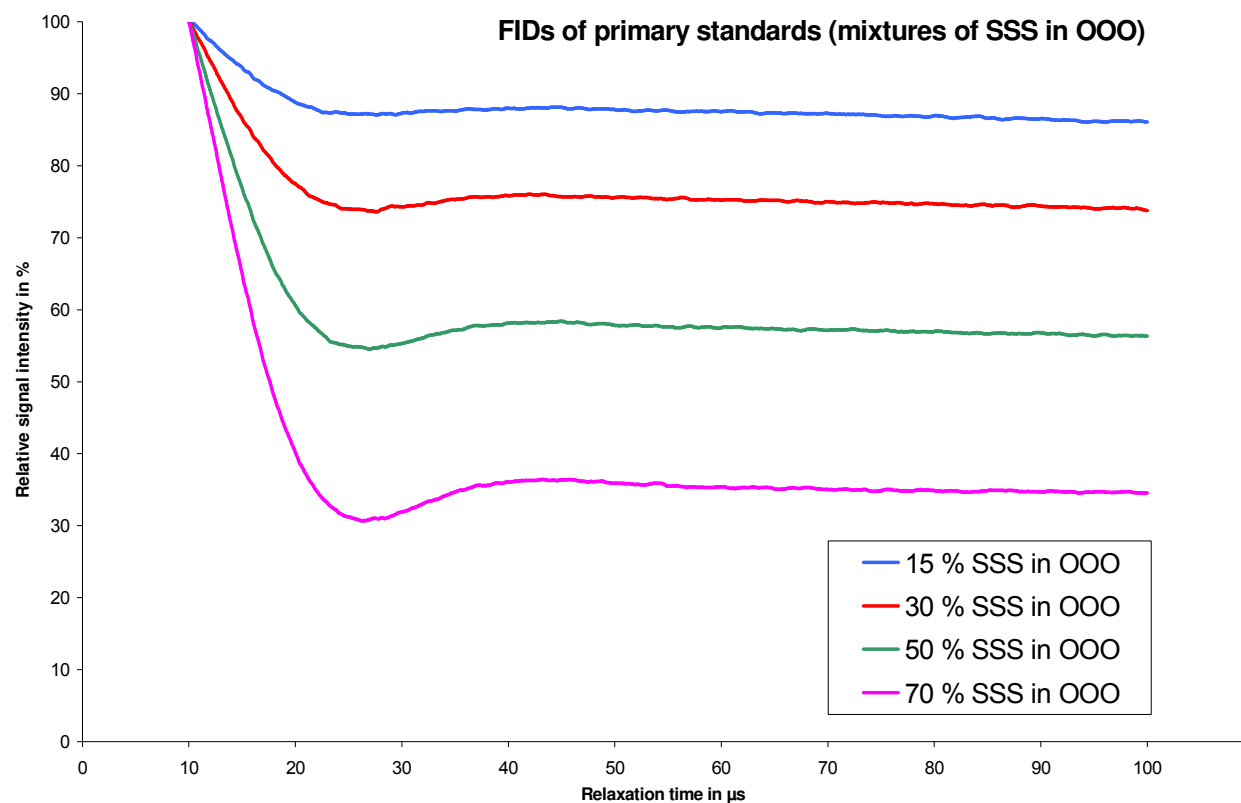


Figure 1: Examples of free induction decays of triglyceride samples with different solid fat contents

<div>Start on Monday</div> <div></div>	<div>Start on Wednesday</div> <div></div>	00h00	Thermostat	80 °C	10 ± 5 min	
		00h10	Homogenize			
		00h10	Thermostat	80 °C	5 min	
		00h15	Thermostat	60 °C	5 min	
		00h20	Cryostat	0 °C	90 ± 5 min	
		01h50	Thermostat	26 °C	40 ± 0.5 h	
<div>Continue on Wednesday</div> <div></div>	<div>Continue on Friday</div> <div></div>	41h50	Cryostat	0 °C	90 ± 5 min	
		43h20	Thermostat	26 °C	60 ± 5 min	
		44h20	Measure			

Figure 2: Timetable model for the tempering scheme to be used for triglyceride standards

Annex B Results of the long-term stability study

Shelf Life and Associated U_{lts}, T=18 °C(Ref)

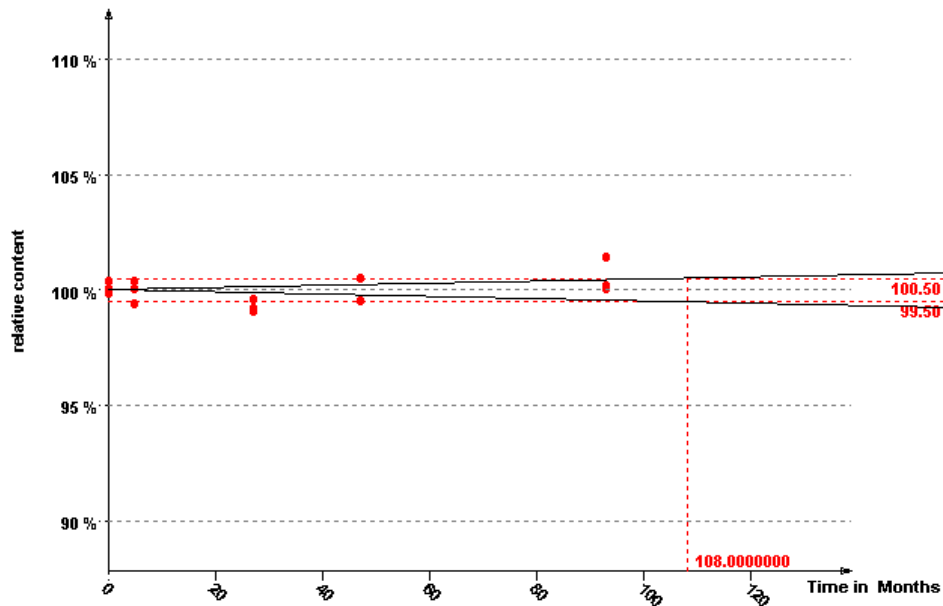


Figure B1: Long-term stability of the F-factor for TGS-01 at 18 °C with associated u_{lts} for a storage period of 108 months

Shelf Life and Associated U_{lts}, T=18 °C(Ref)

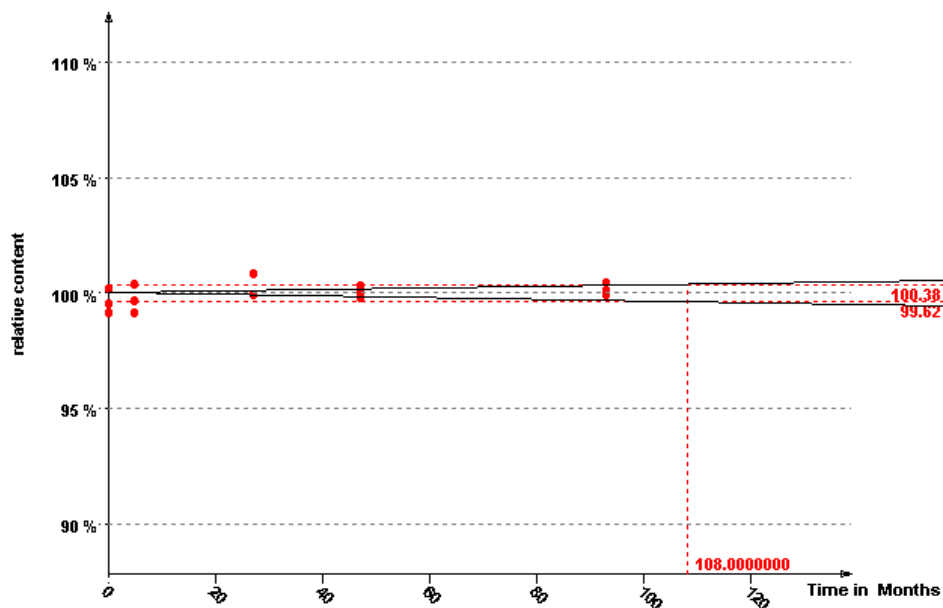


Figure B2: Long-term stability of the F-factor for TGS-02 at 18 °C with associated u_{lts} for a storage period of 108 months

Shelf Life and Associated Ults, T=18 °C(Ref)

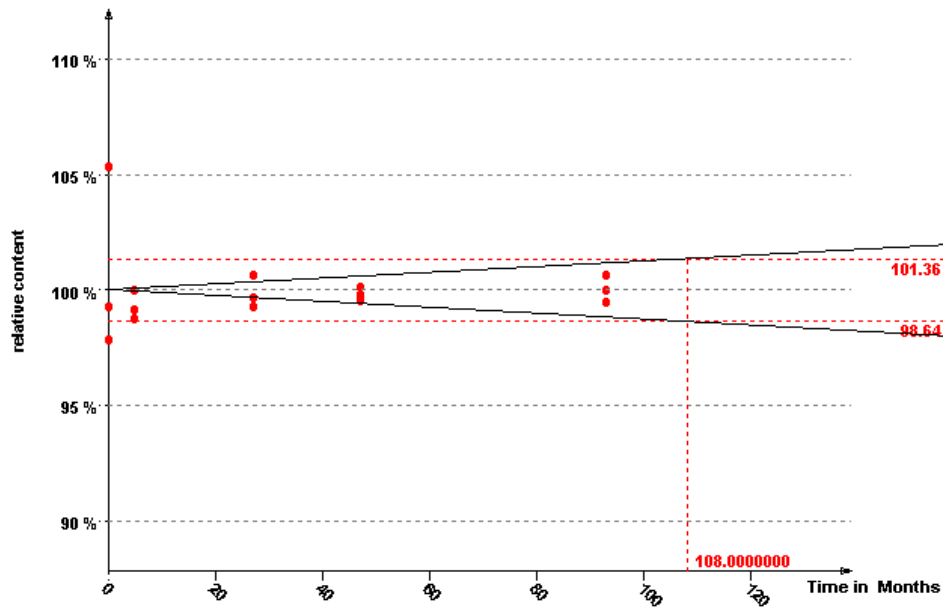


Figure B3: Long-term stability of the F-factor for TGS-03 at 18 °C with associated u_{lts} for a storage period of 108 months

Shelf Life and Associated Ults, T=18 °C(Ref)

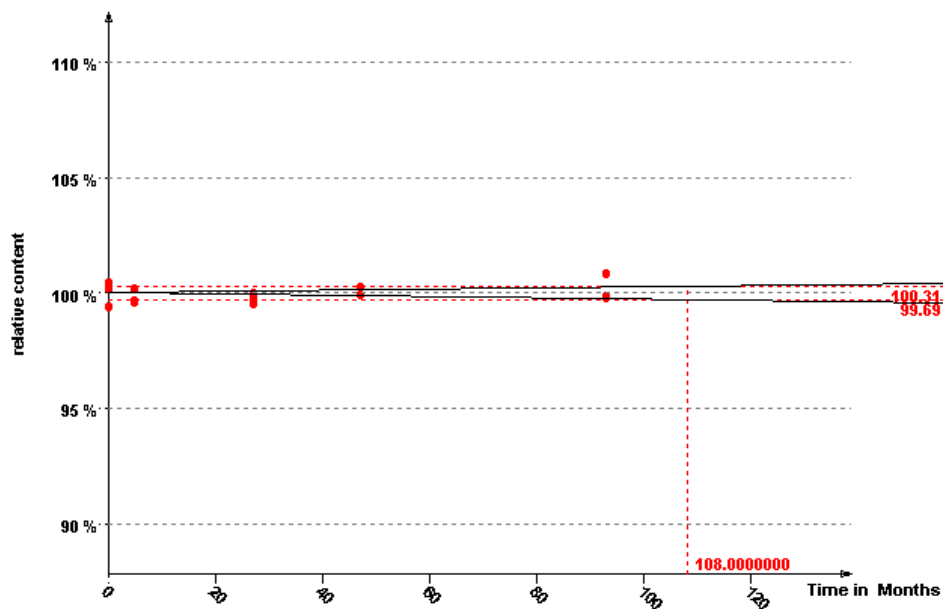


Figure B4: Long-term stability of the F-factor for TGS-04 at 18 °C with associated u_{lts} for a storage period of 108 months

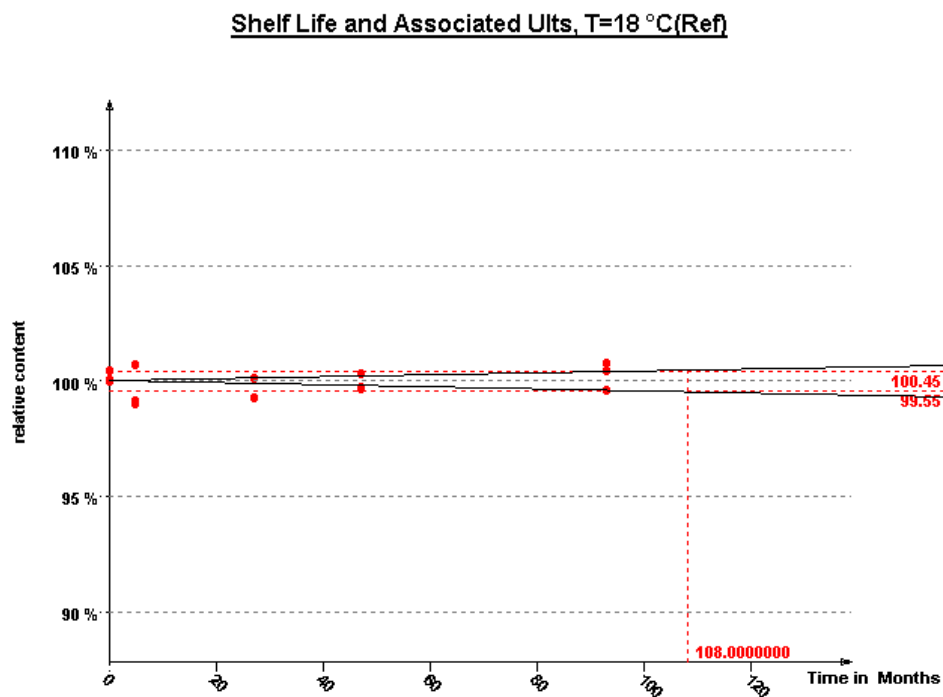


Figure B5: Long-term stability of the F-factor for TGS-05 at 18 °C with associated u_{ITS} for a storage period of 108 months

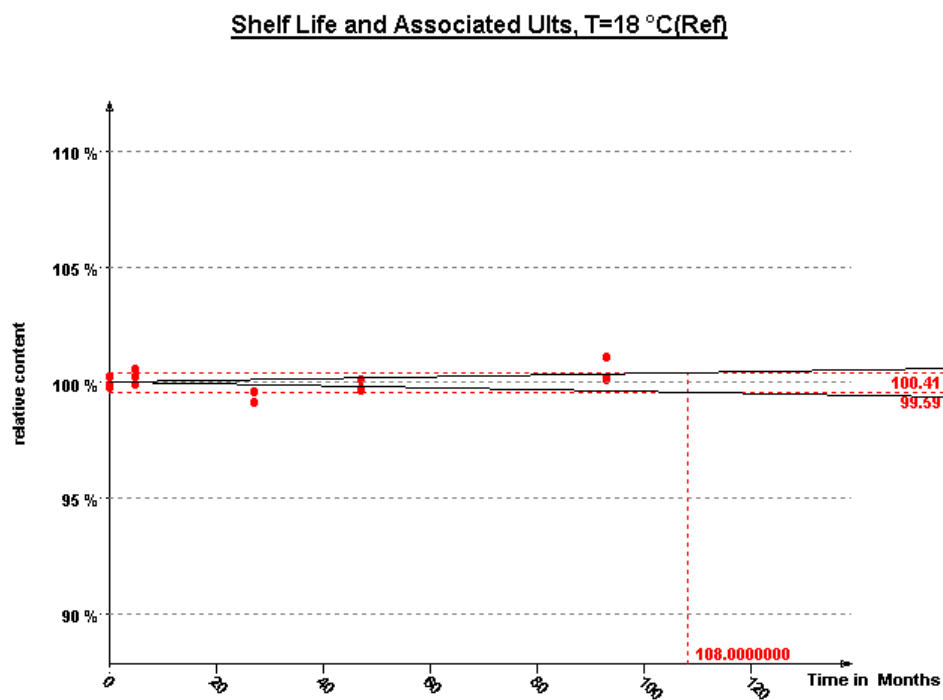


Figure B6: Long-term stability of the F-factor for TGS-06 at 18 °C with associated u_{ITS} for a storage period of 108 months

Shelf Life and Associated Ults, T=18 °C(Ref)

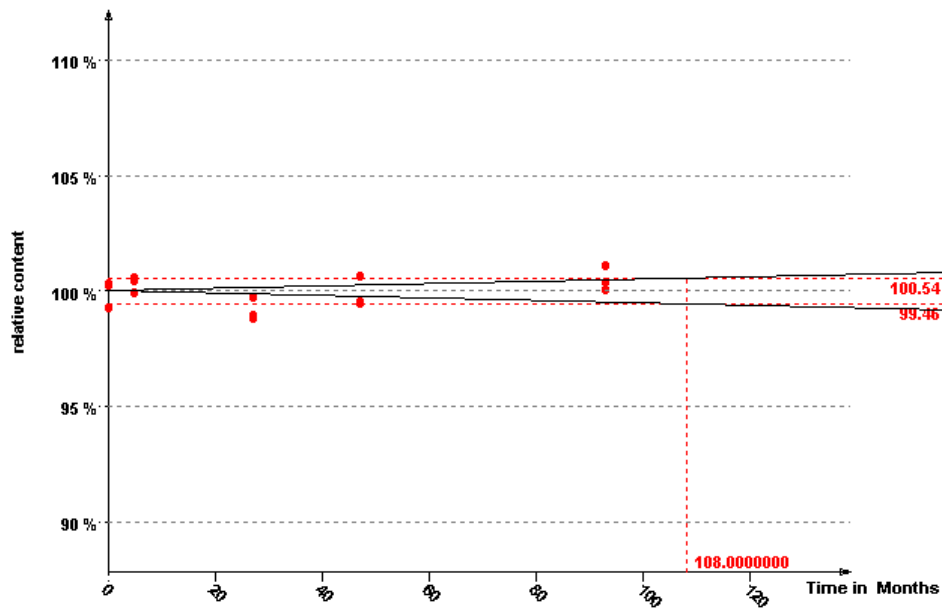


Figure B7: Long-term stability of the F-factor for TGS-07 at 18 °C with associated u_{ITS} for a storage period of 108 months

Shelf Life and Associated Ults, T=18 °C(Ref)

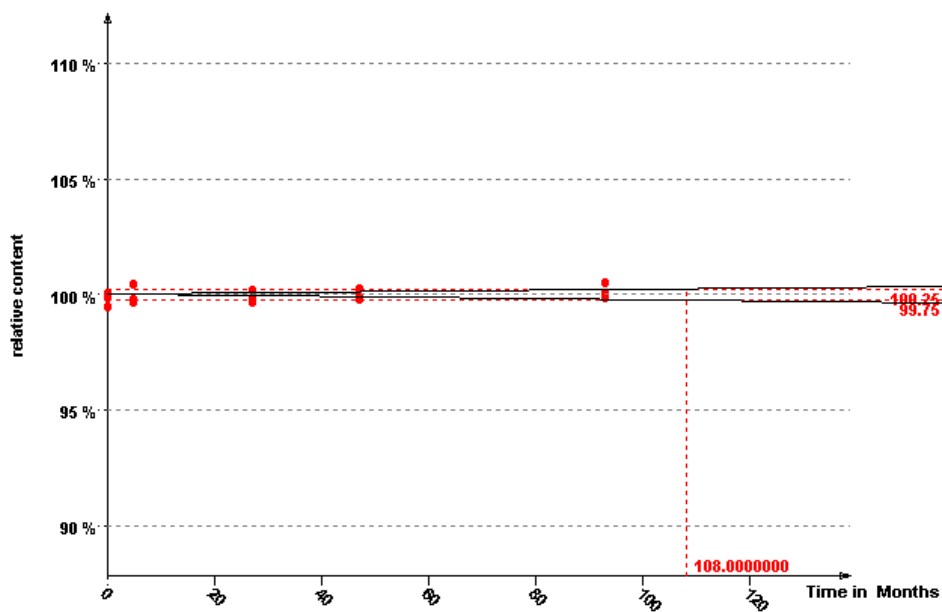


Figure B8: Long-term stability of the F-factor for TGS-08 at 18 °C with associated u_{ITS} for a storage period of 108 months

Shelf Life and Associated U_{lts}, T=18 °C(Ref)

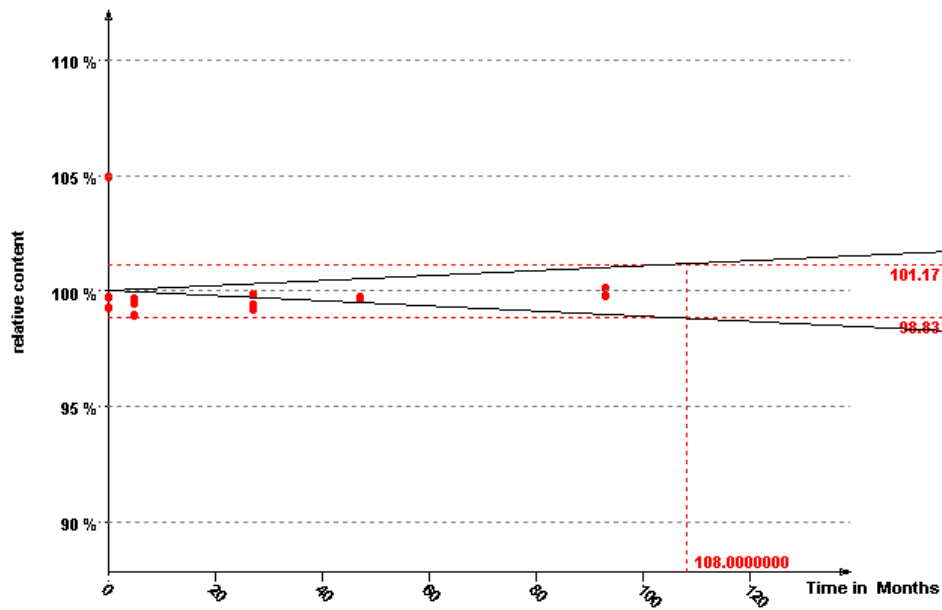


Figure B9: Long-term stability of the F-factor for TGS-09 at 18 °C with associated u_{lts} for a storage period of 108 months

Shelf Life and Associated U_{lts}, T=18 °C(Ref)

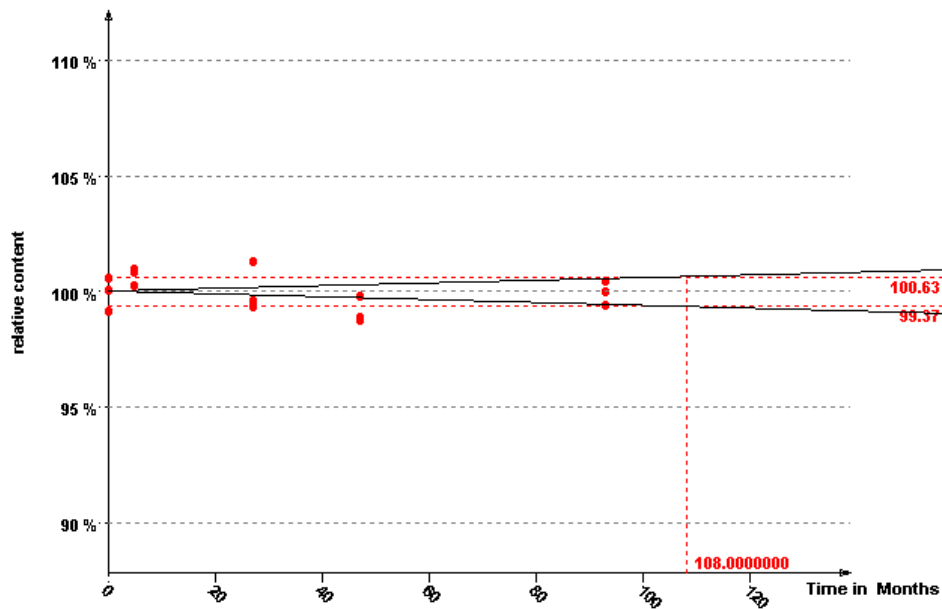


Figure B10: Long-term stability of the F-factor for TGS-10 at 18 °C with associated u_{lts} for a storage period of 108 months

Shelf Life and Associated U_{lts}, T=18 °C(Ref)

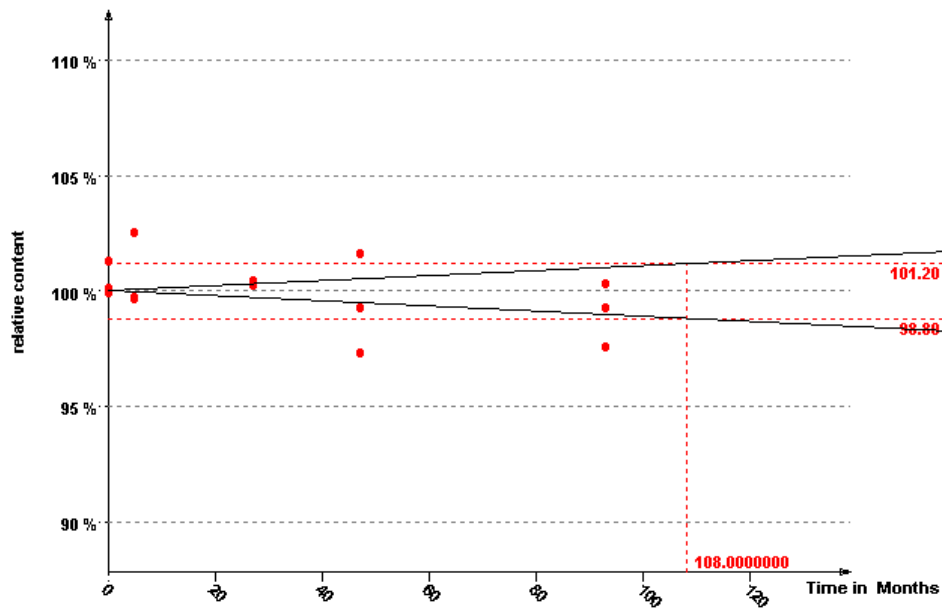


Figure B11: Long-term stability of the F-factor for TGS-11 at 18 °C with associated u_{lts} for a storage period of 108 months

Shelf Life and Associated U_{lts}, T=18 °C(Ref)

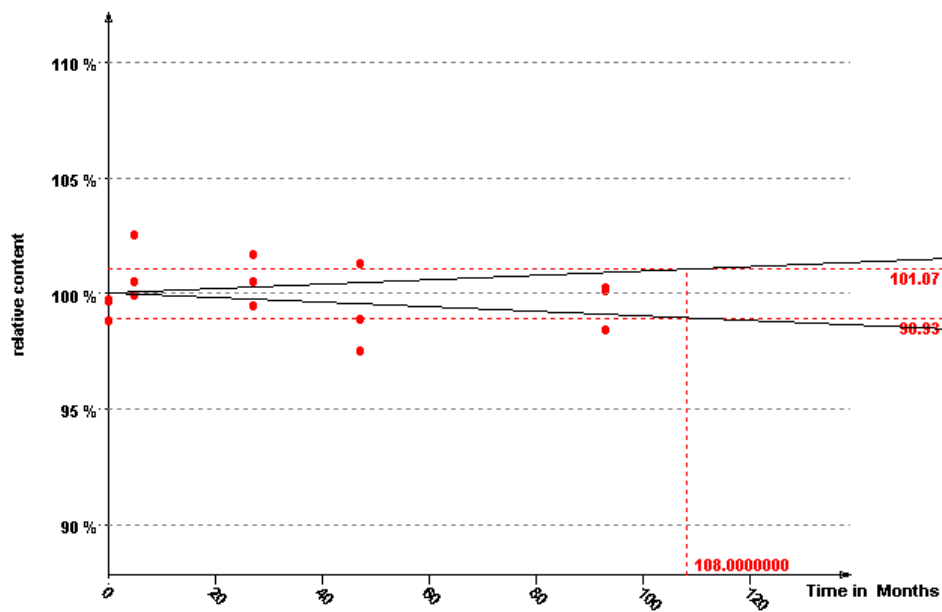


Figure B12: Long-term stability of the F-factor for TGS-12 at 18 °C with associated u_{lts} for a storage period of 108 months

Annex C Characterisation data

Table C1: Results of the characterisation study (F-factor) for TGS-01

F-factor for TGS-01 (SFC = 70.54 %)			
Lab code	Day 1	Day 2	Day 3
1	1.636	1.626	1.618
2	1.598	1.606	1.605
3	1.533	1.589	1.534
4	1.519	1.534	1.530
5	1.532	1.503	1.523
6	1.537	1.560	1.532
7	1.558	n.r.	1.555

n.r. = not reported

Lab Means and their StDev for TGS-01(70%)

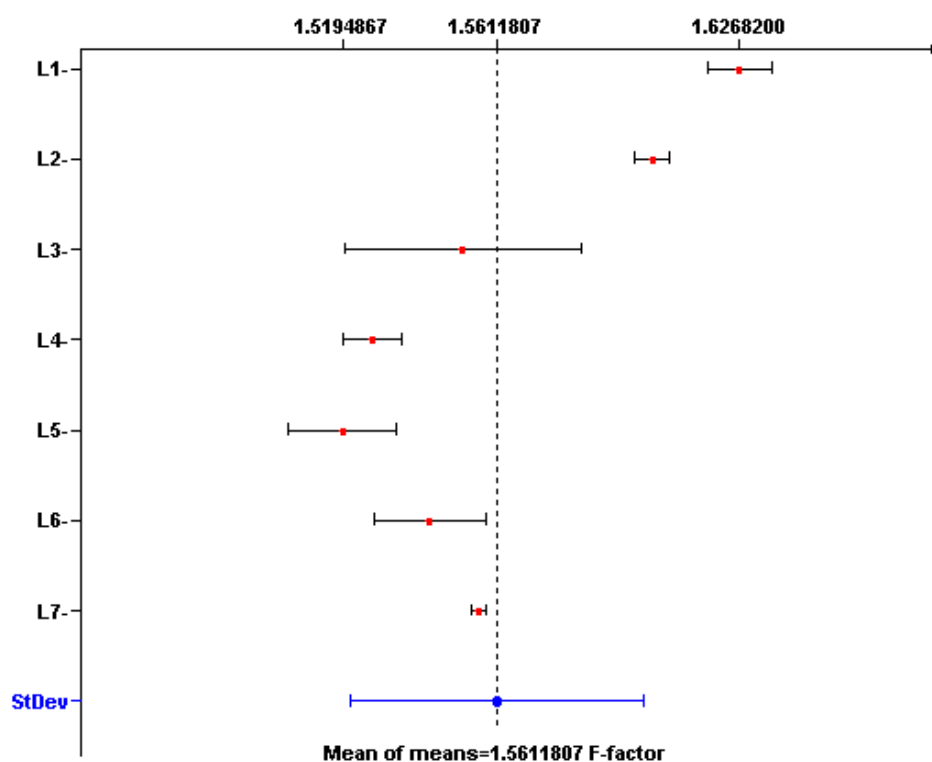


Figure C1: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-01

Table C2: Results of the characterisation study (F-factor) for TGS-02

F-factor for TGS-02 (SFC = 29.22 %)			
Lab code	Day 1	Day 2	Day 3
1	1.631	1.610	1.620
2	1.634	1.622	1.605
3	1.553	1.537	1.546
4	1.546	1.538	1.557
5	1.533	1.494	1.519
6	1.546	1.547	1.547
7	1.568	1.565	1.579

Lab Means and their StDev for TGS-02(30%)

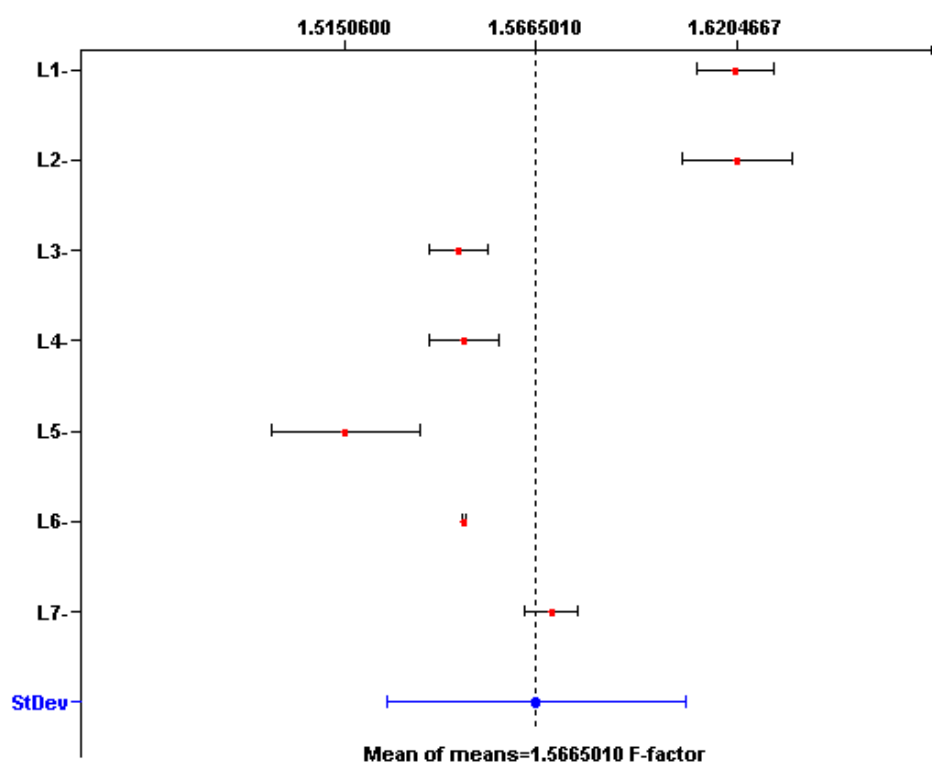


Figure C2: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-02

Table C3: Results of the characterisation study (F-factor) for TGS-03

F-factor for TGS-03 (SFC = 30.51 %)			
Lab code	Day 1	Day 2	Day 3
1	1.704	1.639	1.627
2	1.639	1.613	1.601
3	1.537	1.536	1.556
4	1.541	1.547	1.561
5	1.536	1.495	1.539
6	1.552	1.542	1.556
7	1.573	1.578	1.565

Lab Means and their StDev for TGS-03(30%)

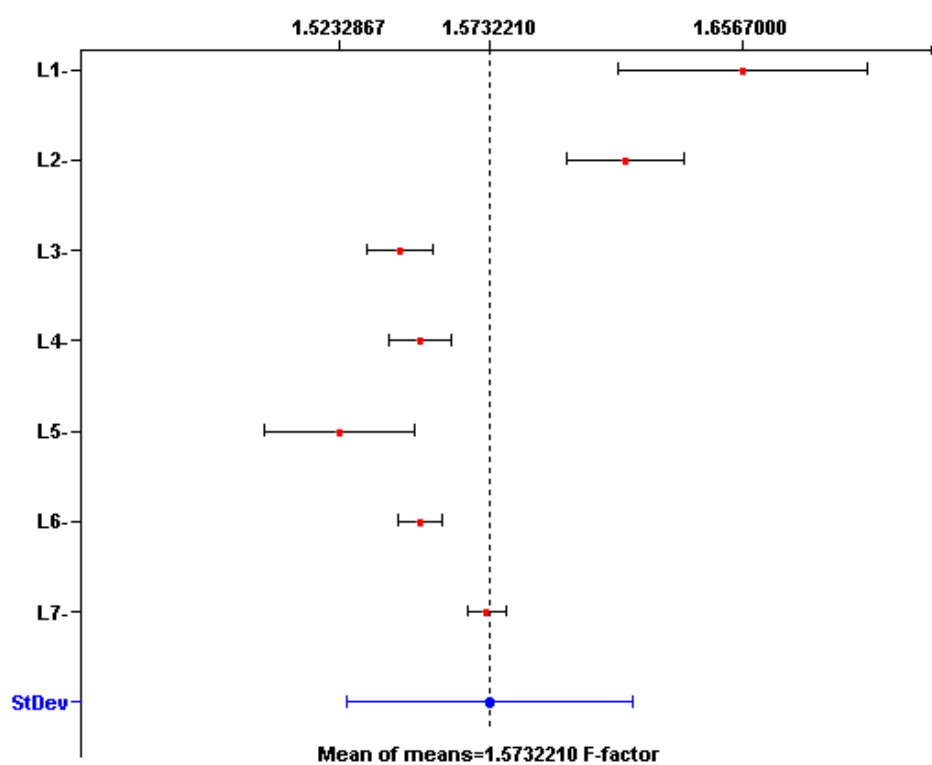


Figure C3: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-03

Table C4: Results of the characterisation study (F-factor) for TGS-04

F-factor for TGS-04 (SFC = 49.70 %)			
Lab code	Day 1	Day 2	Day 3
1	1.739	1.646	1.638
2	1.655	1.612	1.608
3	1.555	1.536	1.541
4	1.545	1.544	1.553
5	1.532	1.515	1.533
6	1.782	1.666	1.605
7	1.557	1.562	1.557

Lab Means and their StDev for TGS-04(50%)

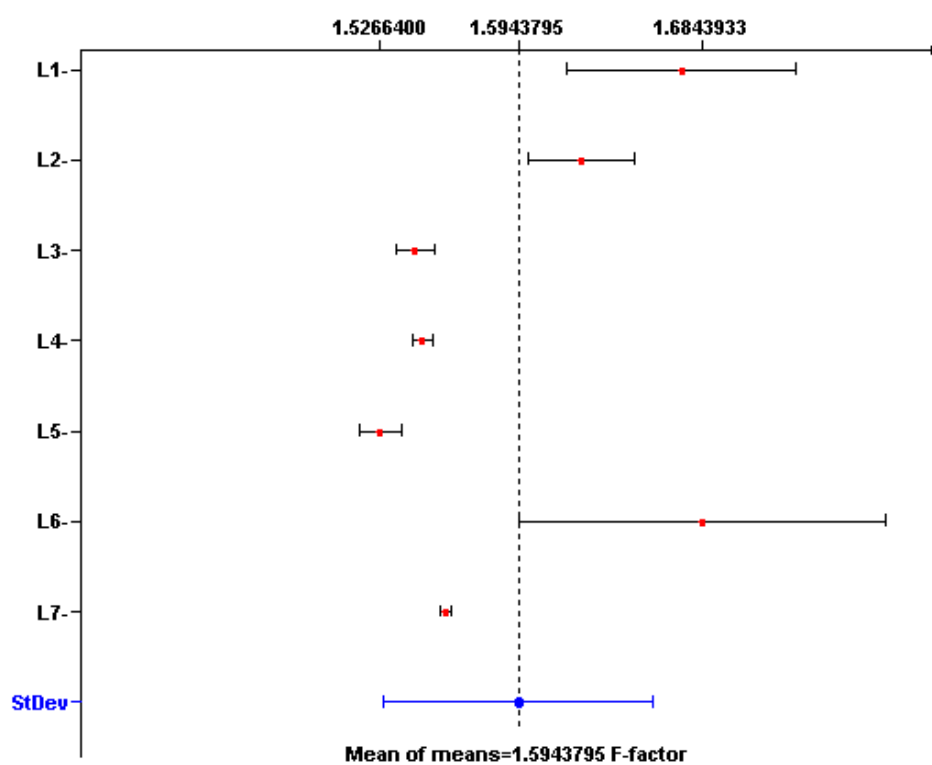


Figure C4: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-04

Table C5: Results of the characterisation study (F-factor) for TGS-05

F-factor for TGS-05 (SFC = 30.09 %)			
Lab code	Day 1	Day 2	Day 3
1	1.648	1.628	1.620
2	1.603	1.615	1.617
3	1.537	1.532	1.547
4	1.541	1.540	1.566
5	1.548	1.520	1.528
6	1.566	1.540	1.543
7	1.558	1.570	1.567

Lab Means and their StDev for TGS-05(30%)

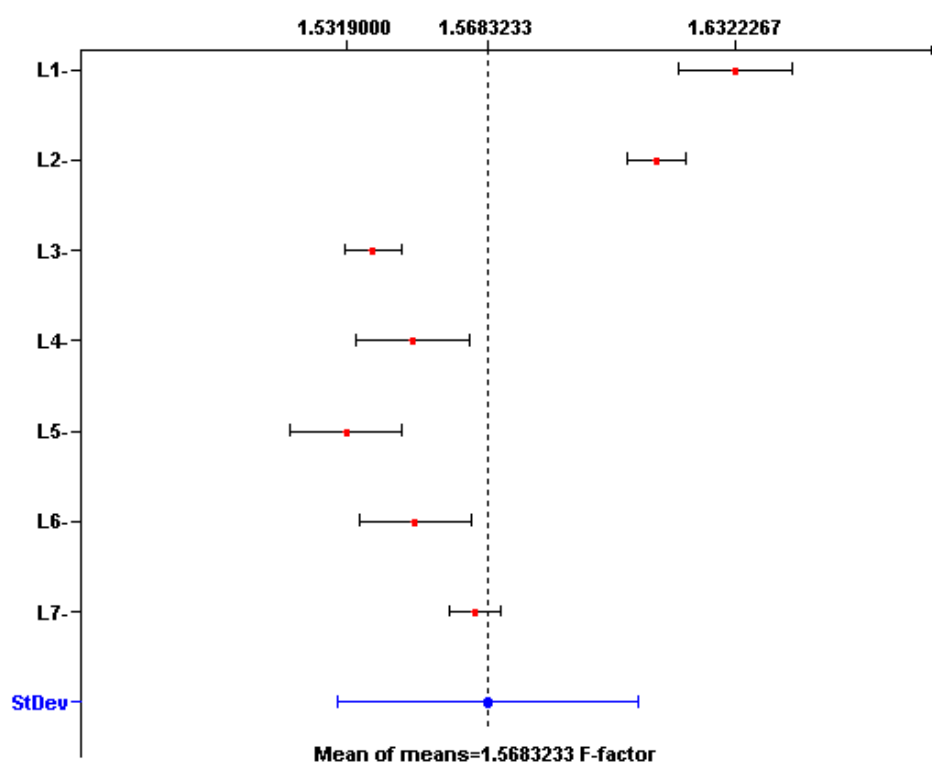


Figure C5: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-05

Table C6: Results of the characterisation study (F-factor) for TGS-06

F-factor for TGS-06 (SFC = 69.87 %)			
Lab code	Day 1	Day 2	Day 3
1	1.671	1.629	1.643
2	1.609	1.610	1.605
3	1.548	1.548	1.541
4	1.531	1.536	1.541
5	1.519	1.520	1.500
6	1.567	1.542	1.531
7	1.543	1.640	1.552

Lab Means and their StDev for TGS-06(70%)

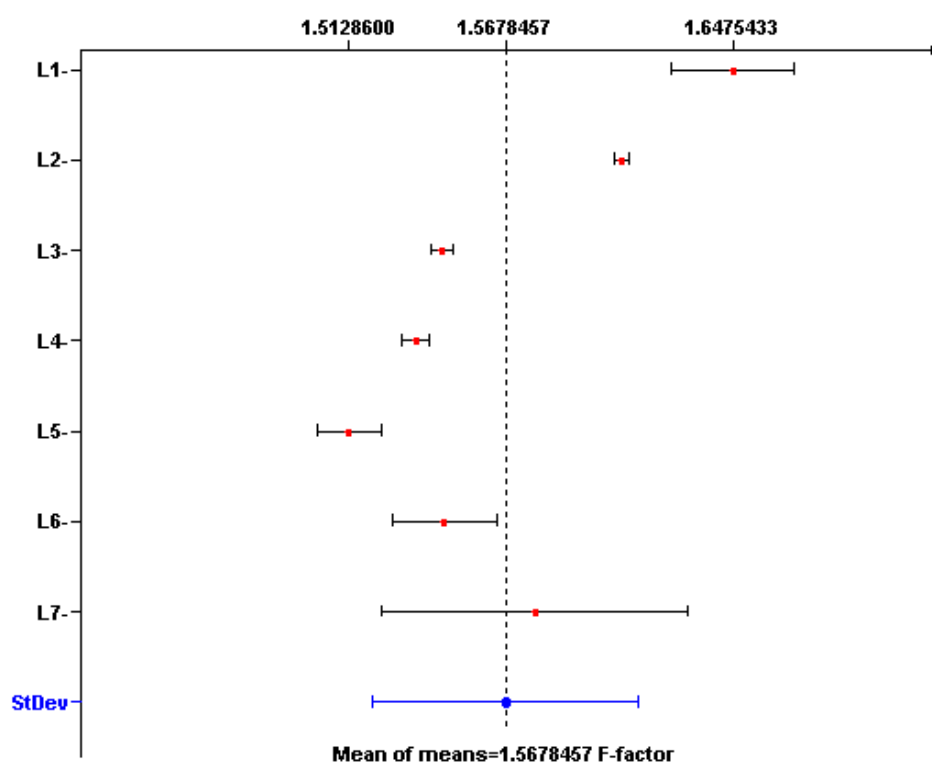


Figure C6: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-06

Table C7: Results of the characterisation study (F-factor) for TGS-07

F-factor for TGS-07 (SFC = 68.87 %)			
Lab code	Day 1	Day 2	Day 3
1	1.668	1.628	1.641
2	1.620	1.611	1.600
3	1.537	1.542	1.536
4	1.528	1.536	1.538
5	1.513	1.520	1.506
6	1.554	1.533	1.536
7	1.631	1.662	1.555

Lab Means and their StDev for TGS-07(70%)

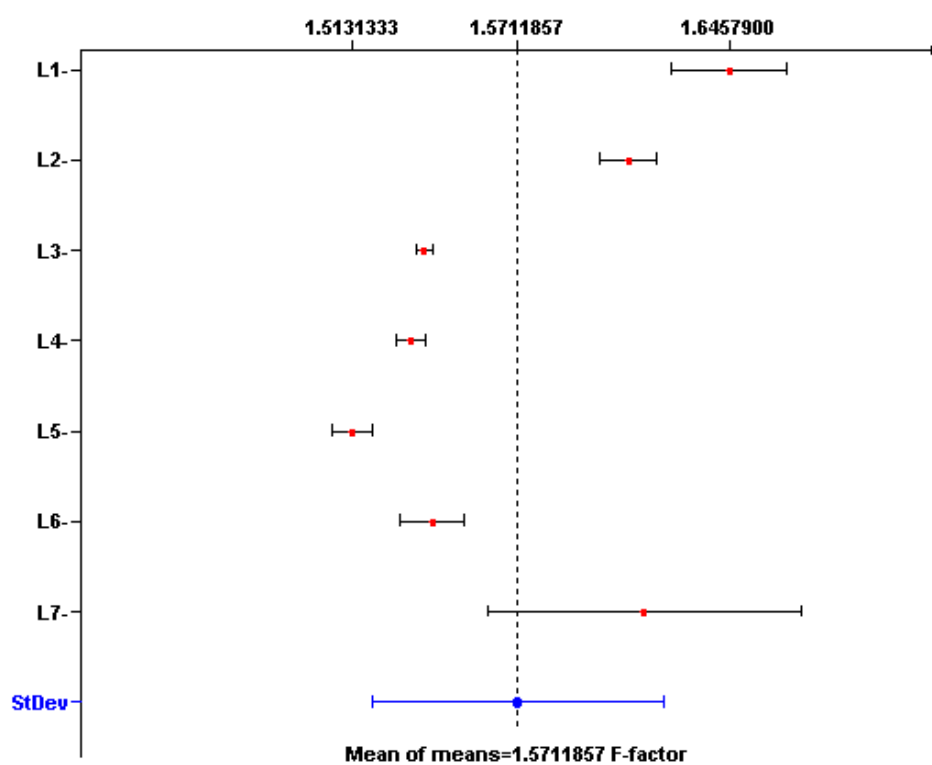


Figure C7: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-07

Table C8: Results of the characterisation study (F-factor) for TGS-08

F-factor for TGS-08 (SFC = 49.49 %)			
Lab code	Day 1	Day 2	Day 3
1	1.722	1.633	1.637
2	1.654	1.625	1.602
3	1.538	1.538	1.533
4	1.542	1.543	1.554
5	1.526	1.527	1.525
6	1.663	1.619	1.564
7	1.555	1.557	1.559

Lab Means and their StDev for TGS-08(50%)

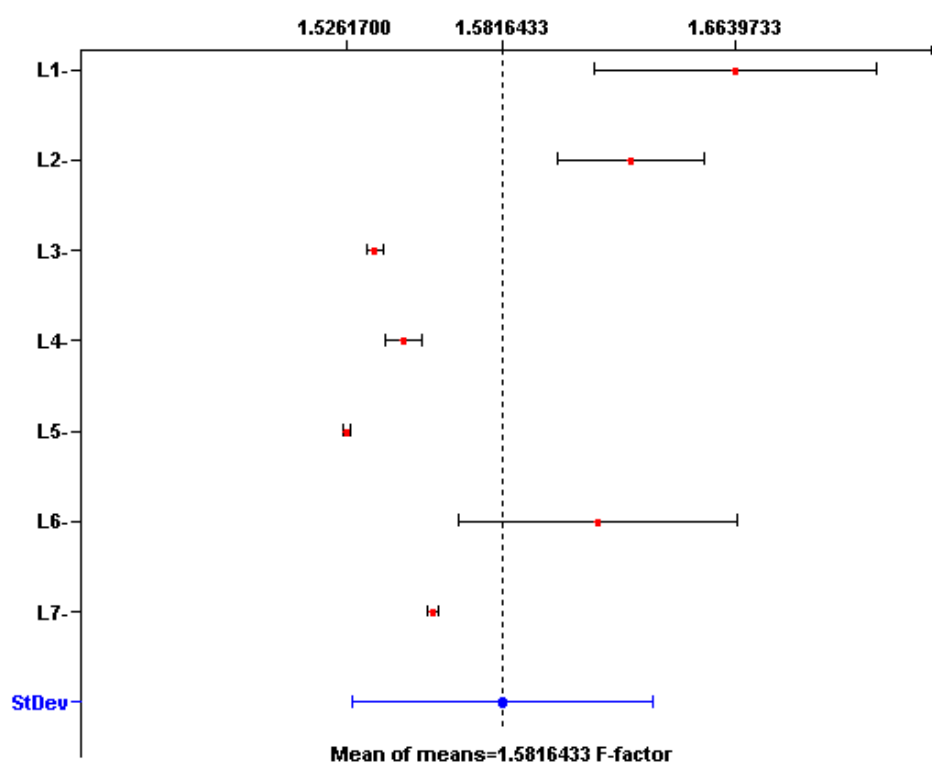


Figure C8: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-08

Table C9: Results of the characterisation study (F-factor) for TGS-09

F-factor for TGS-09 (SFC = 50.00 %)			
Lab code	Day 1	Day 2	Day 3
1	1.739	1.614	1.632
2	1.621	1.616	1.609
3	1.551	1.548	1.536
4	1.538	1.546	1.549
5	1.520	1.530	1.515
6	1.672	1.608	1.563
7	1.564	1.570	1.555

Lab Means and their StDev for TGS-09(50%)

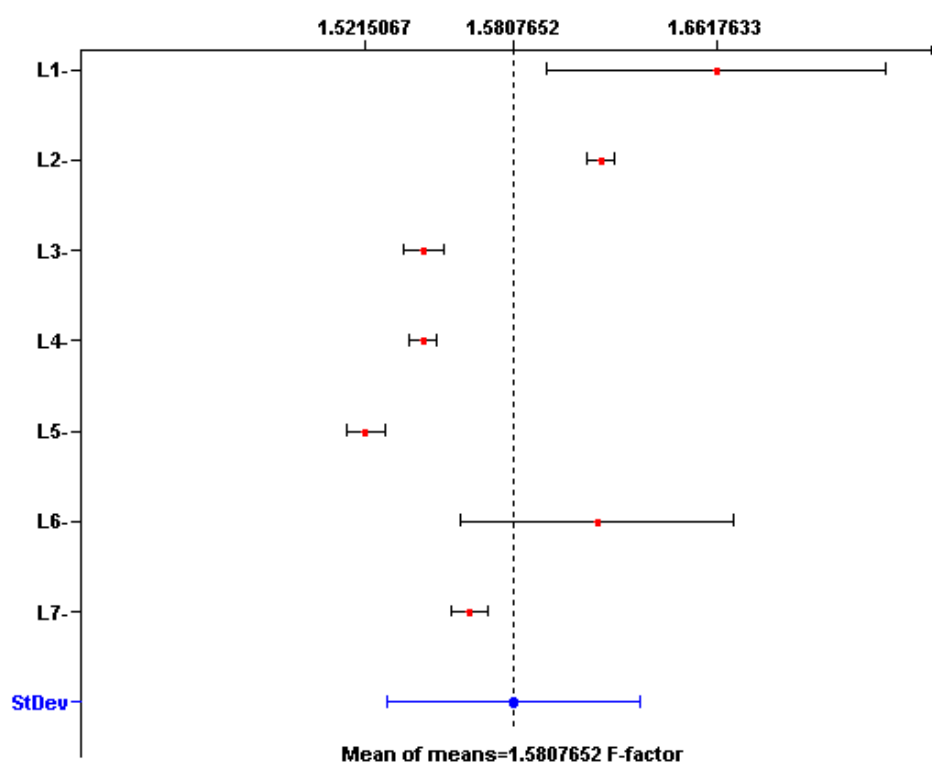


Figure C9: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-09

Table C10: Results of the characterisation study (F-factor) for TGS-10

F-factor for TGS-10 (SFC = 14.95 %)			
Lab code	Day 1	Day 2	Day 3
1	1.653	1.594	1.624
2	1.603	1.607	1.629
3	1.507	1.551	1.549
4	1.565	1.556	1.566
5	1.512	1.515	1.528
6	1.547	1.537	1.562
7	1.596	1.564	1.578

Lab Means and their StDev for TGS-10(15%)

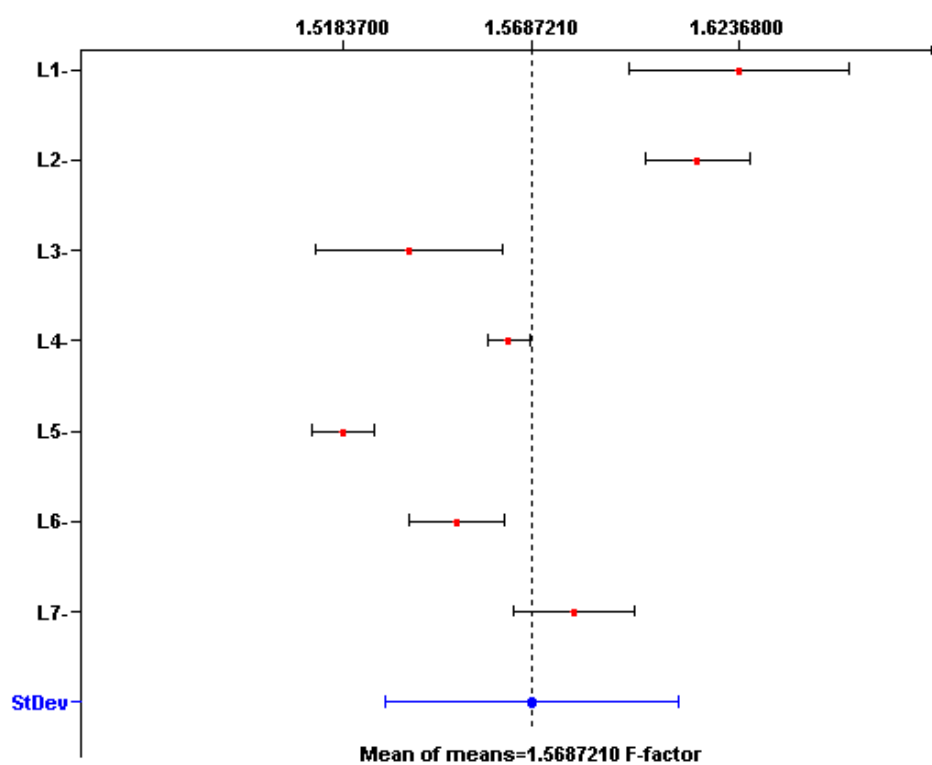


Figure C10: Laboratory means, mean of means and corresponding standard deviations (F-factor) for TGS-10

Table C11: Results of the characterisation study (F-factor) for TGS-11

F-factor for TGS-11 (SFC = 14.95 %)			
Lab code	Day 1	Day 2	Day 3
1	1.619	1.657	1.636
2	1.640	1.628	1.605
3	1.504	1.565	1.553
4	1.557	1.556	1.601
5	1.523	1.541	1.539
6	1.562	1.537	1.540
7	1.588	1.558	1.566

Lab Means and their StDev for TGS-11(15%)

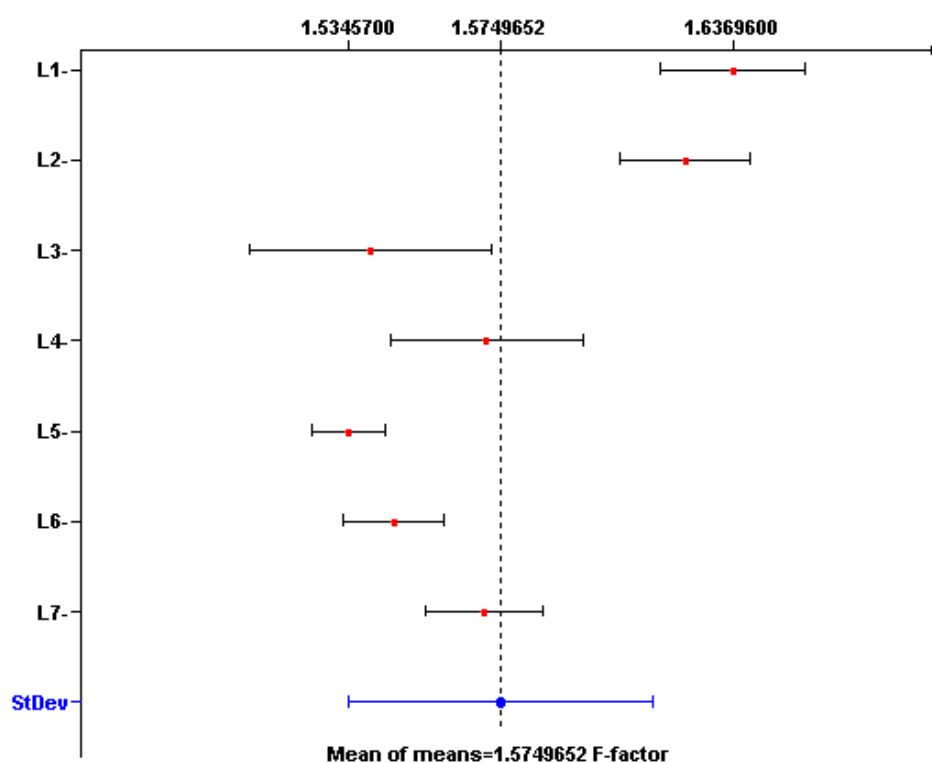


Figure C11: Laboratory means, mean of means and corresponding standard deviations (F-Factor) for TGS-11

Table C12: Results of the characterisation study (F-factor) for TGS-12

F-factor for TGS-12 (SFC = 15.04 %)			
Lab code	Day 1	Day 2	Day 3
1	1.632	1.630	1.675
2	1.607	1.612	1.600
3	1.509	1.544	1.547
4	1.551	1.560	1.591
5	1.520	1.530	1.520
6	1.535	1.547	1.544
7	1.552	1.589	1.559

Lab Means and their StDev for TGS-12(15%)

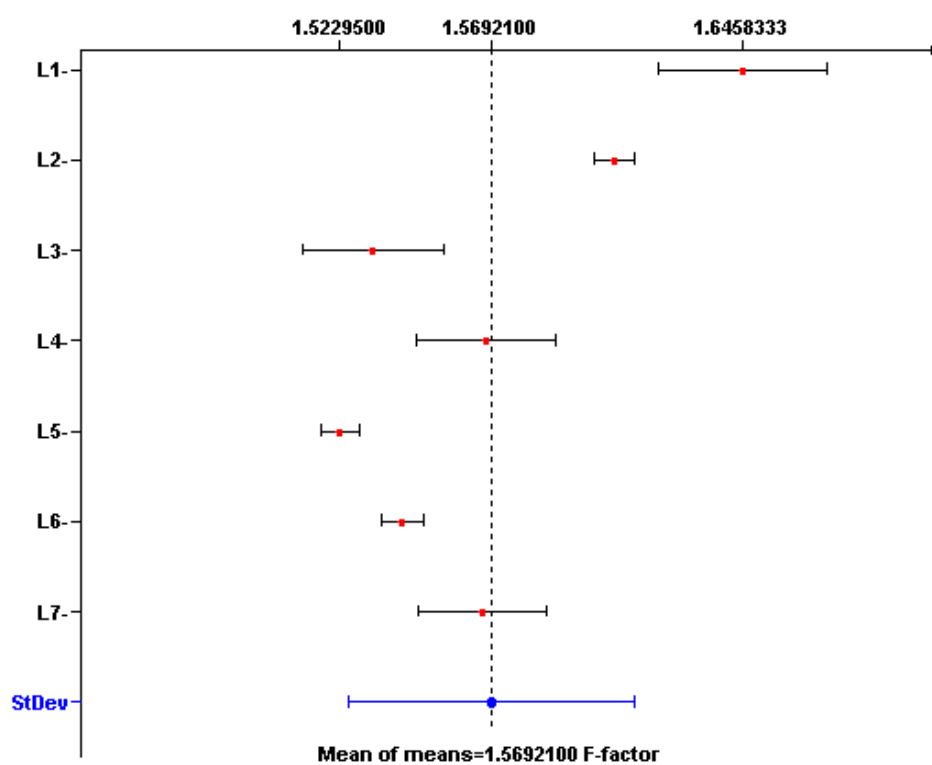


Figure C12: Laboratory means, mean of means and corresponding standard deviations (F-Factor) for TGS-12

EUR 24959 EN – Joint Research Centre – Institute for Reference Materials and Measurements

Title: The Certification of the Mass Fractions of Tristearin in Blends of Pure Tristearin and Triolein Representing the Solid Fat Content - IRMM-805 (Set of 12 Primary Standards)

Author(s): A. Bernreuther, H. Schimmel

Luxembourg: Publications Office of the European Union

2011 – 36 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online), 1018-5593 (print)

ISBN 978-92-79-21470-7 (pdf)

ISBN 978-92-79-21469-1 (print)

doi:10.2787/53378

Abstract

This report describes the preparation and certification of IRMM-805. This reference material is comprised of a set of 12 tristearin/triolein blends (primary triglyceride samples) for the solid fat content (SFC) determination by low-resolution nuclear magnetic resonance spectrometry (LR-NMR).

The preparation and processing of the materials, stability studies and a characterisation study are described hereafter and the results are discussed. Uncertainties were estimated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties due to gravimetric sample preparation, purity and possible instability.

How to obtain EU publications

Our priced publications are available from EU Bookshop (<http://bookshop.europa.eu>), where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents. You can obtain their contact details by sending a fax to (352) 29 29-42758.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

